Supporting Information

Fabrication of human serum albumin–imprinted pthotothermal nanoparticle for enhanced immunotherapy

Ma, JZ(Ma, Jizhuang)[1] ; Zhang, Y(Zhang, Yao)[1] ; Sun, HY(Sun, Hongyi)[1] ;

Ding, PT(Ding, Pingtian)*[1] ; Chen, DW (Chen, Dawei)*[1]

[1] School of Pharmacy, Department of Pharmaceutics, Shenyang Pharmaceutical

University, No. 103, Wenhua Road, Shenyang 110016, PR China

A: Experimental Section

1. Materials and Methods

1. Materials

Fe_3O_4 nanoparticles, dopamine, indocyanine green-SH (ICG-SH) were obtained from Aladdin

Industrial Co., Ltd. (Shanghai, China); human serum album (HSA), bovine hemoglobin

(BHb), immunoglobulin G (Ig G), fibrinogen (Fib), fluorescein isothiocyanate (FITC) were

obtained from Meilun Biotechnology (Dalian, China).

2. Characterization of the nanoparticles

The shape and surface morphology of the particles was observed by the Transmission

Electron Microscopy (TEM, Tecnai G2 F30, FEI Company, Hillsboro, Oregon, USA). To

prepare samples for TEM, samples were dissolved in water at a concentration of 0.1 mg/mL

and ultrasonicated for 10 min. Then a drop of the sample solution was placed on a carbon-

coated copper grid and dried in air for at least 24 h. The size and zeta potential were measured

by Malvern Mastersizer 2000 (Zetasizer Nano ZS90, Malvern Instruments Ltd., UK). FT-IR

spectra were recorded in the range between 4000 and 400 cm^{-1} on a FT-IR spectrophotometer (Thermo Nicolet, Madison, Wisconsin). The thermal profile of the particles was measured by TGA (Netzsch STA 449, Germany), which can show the weight loss pattern of each sample. 10 mg particles were loaded in thermal gravimetric analyzer in an air atmosphere and heated to 800 °C with a rate of 10 °C min^{-1}.

3. Protein adsorption formula

The adsorption capacity (Q) was calculated from the following formula:

$$Q = (C_0 - C)V/m \tag{1}$$

where Q (mg ml^{-1}) is the adsorption capacities of protein on a unit amount of dried nanoparticles, C_0 (mg ml^{-1}) is the initial HSA concentration, C (mg ml^{-1}) is the HSA concentration in the supernatant after adsorption, V (ml) is the volume of HSA solution and m (g) is the mass of Fe_3O_4@MIPs nanoparticles or Fe_3O_4@NIPs nanoparticles.

The imprinting factor (α) was calculated from the following formula:

$$\alpha = Q_{MIP}/Q_{NIP} \tag{2}$$

where Q_{MIP} and Q_{NIP} are the adsorption amount of proteins on Fe_3O_4@MIPs and Fe_3O_4@NIPs, respectively.

4. Liquid-chromatography mass-spectrometry (LC-MS/MS) analysis of protein corona

Gel bands were cut into 1 mm pieces and washed with 50% ACN solution containing 25 mM ammonium bicarbonate (ABC) to remove the stain. After washing, the samples were reduced and alkylated. Sequence grade Lys-C/Trypsin (Promega) was used to enzymatically digest the gel bands. All digestions were carried out in the Barocycler NEP2320 (Pressure Biosciences, Inc.) at 50 °C under 20,000 psi for 1 h. Peptides were recovered from the gel samples using

60% ACN/5% trifluoroacetic acid (TFA)/35% DI water with sonication in an ice bath. The supernatant was removed from the gel and placed in a vacuum centrifuge to dry. The resulting pellets were resuspended in 97% DI water/3% ACN/0.1% formic acid (FA) prior to LC-MS/MS analysis.

Samples were analyzed using the Dionex UltiMate 3000 RSLC Nano System coupled to the Q Exactive™ HF Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, Waltham, MA). Peptides were loaded onto a C18 PepMap™ 100 trap column (300 μm i.d. × 5 mm) and washed at a flow rate of 5 μL/min with 98% purified water/2% ACN/0.01% FA. The trap column was switched in-line with the analytical column after 5 min, and peptides were separated by a reverse phase Acclaim™ PepMap™ RSLC C18 (75 μm × 15 cm) analytical column using a 120 min method at a flow rate of 300 nL/min. Mobile phase A was 0.01% FA in water, and mobile phase B was 0.01 % FA in 80% ACN. The linear gradient started at 5% B and reached 30% B in 80 min, 45% B in 91 min, and 100% B in 93 min. The column was held at 100% B for the next 5 min and returned to 5% B and held for 20 min. Samples were injected in a random order to the QE HF through the Nanospray Flex™ Ion Source fitted with an emission tip from Thermo Scientific. Data acquisition was performed monitoring the top 20 precursors at 120,000 resolution with an injection time of 100 msec. The peptide masses were searched against a human protein sequence database (Uniprot) using the Mascot Daemon v.2.5.1 (Matrix Science), with peptide mass tolerance of 0.05 Da, fragment mass tolerance of 0.2 Da, 1% false discovery rate (FDR), trypsin digestion, carbamidomethyl cysteine as fixed modification, and oxidized methionine as variable modification. Proteins satisfying the following criteria were selected: (i) protein mass <333

kDa, (ii) exponentially modified protein abundance index (emPAI) >1.0, (iii) spectral count >100, and (iv) peptide count >5. Contaminants (e.g. keratin) were excluded. The percentage of each protein fraction was calculated as the spectral count of individual protein divided by the total protein spectral count.

5. Cell Culture

Cells were cultured in a humidified incubator (Thermo Fisher Scientific Inc. USA) with 5% CO_2 at 37 °C in Dulbecco's Modified Eagle Medium (DMEM, GIBCO, Invitrogen) supplemented with 10% FBS, penicillin (100 units mL^{-1}) and streptomycin (100 mg mL^{-1}).

6. Tumor Models

Six-week-old female BALB/c mice were obtained from the Shenyang Pharmaceutical University (Shenyang, China) Experimental Animal Center. To establish the mice tumor model, 4T1 tumors were generated in each mouse by subcutaneous injection of 4T1 cells (1×10^6 cells, 100 μL PBS) in the left flank of mice. The length and width of tumor were measured by digital caliper at predetermined intervals. The tumor volumes were monitored at predetermined intervals using a digital caliper. Tumor volume (V) was calculated according to the following formula: tumor volume $= L \times W^2/2$, where L is the length and W is the width. All animal studies were performed in line with the guidelines for animal experimentation of Shenyang Pharmaceutical University and obtained approval for the animal experiments from the Ethics Committee of the institution (SYPHUIACUC-C2021-5-15-105).

7. Biodistribution of Fe_3O_4@MIPs and Fe_3O_4@NIPs

The 4T1 tumor-bearing mice were intravenously (i.v.) administrated with Fe_3O_4@MIPs and Fe_3O_4@NIPs at a dose of 20 mg/kg. Animals were sacrificed at the indicated time points and

major organs and tumors were collected after 24 h, and the concentration of Fe_3^+ was measured with ICP-OES.

8. Statistical Analysis.

Statistical analysis was performed by one-way ANOVA.
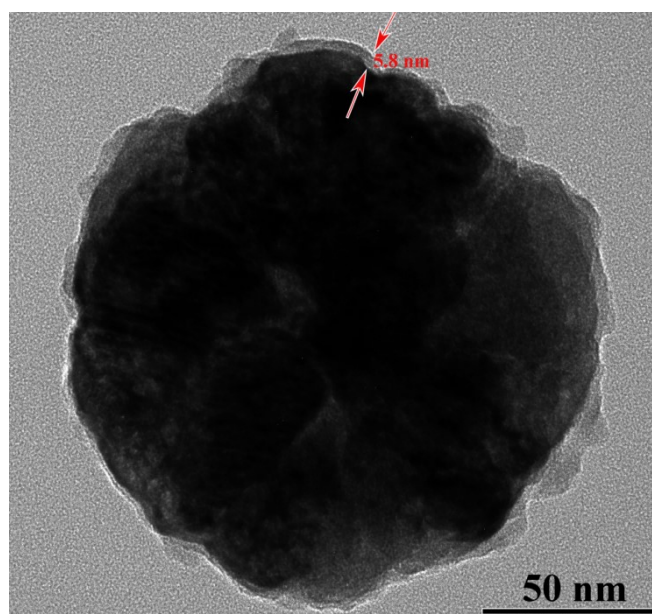
B: Supplementary figures
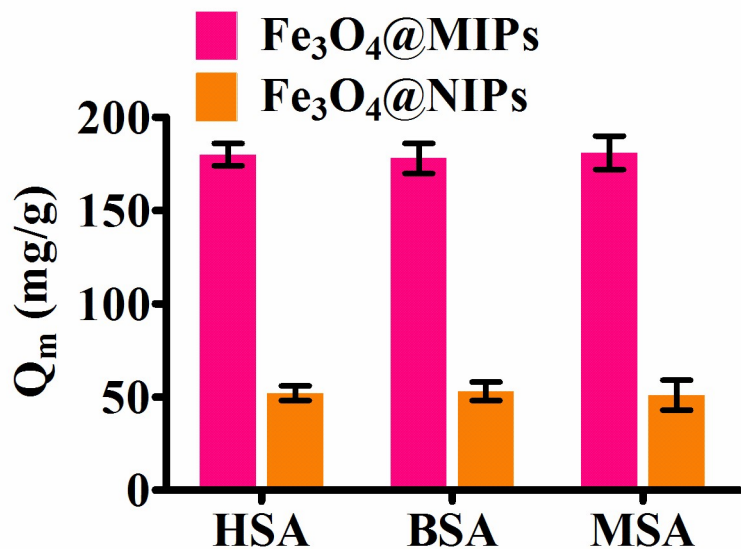

Figure S1. TEM of PDA -Fe_3O_4 NPs.

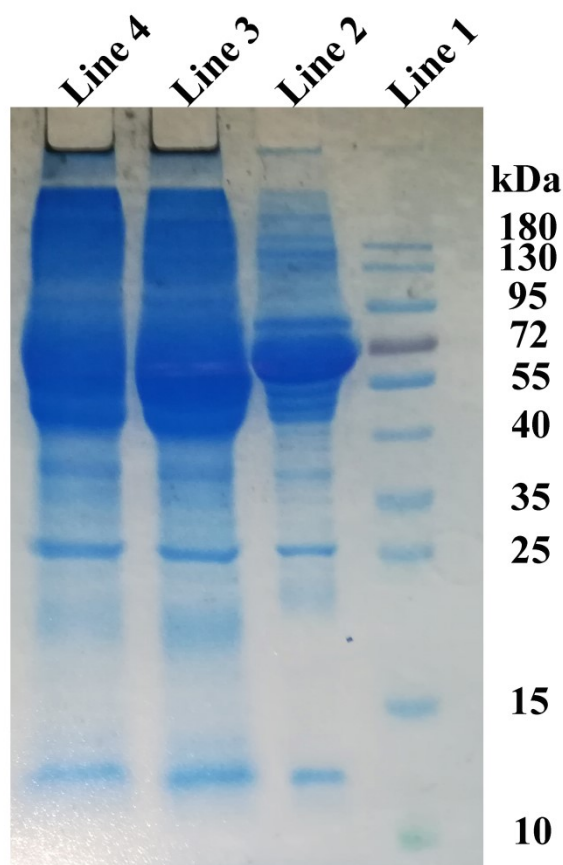Figure S2. Binding amount of Fe_3O_4@MIPs toward HSA, BSA and MSA.

Figure S3. SDS-PAGE analysis of protein corona of Fe_3O_4@MIPs and Fe_3O_4@NIPs after incubating with mouse blood serum. Lane (1)—marker; (2)—10% mouse blood serum ; (3) —the eluate from Fe_3O_4@MIPs;(4) —the eluate from Fe_3O_4@NIPs; loading amount of protein mixtures: 10 ul.
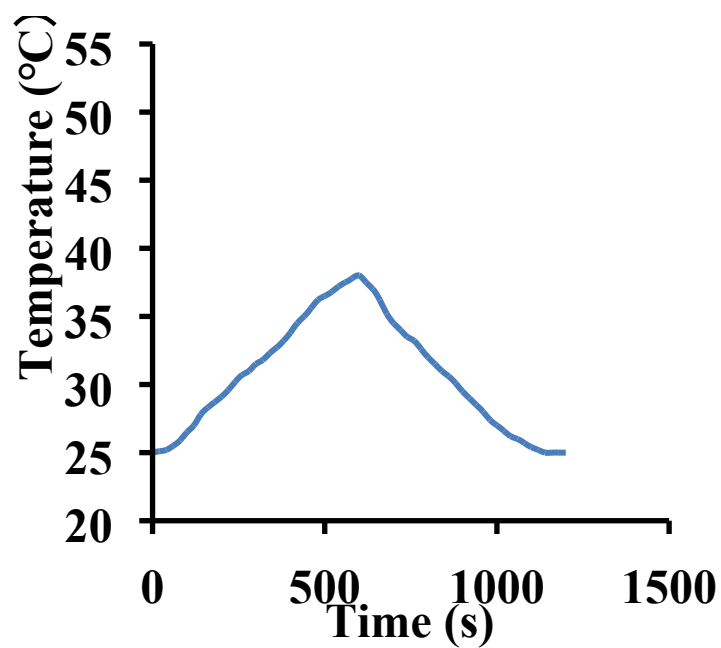
Figure S4. Temperature curves of Fe$_3$O$_4$ NPs at the concentration of 300 µg mL^{-1} as a function of laser irradiation time.
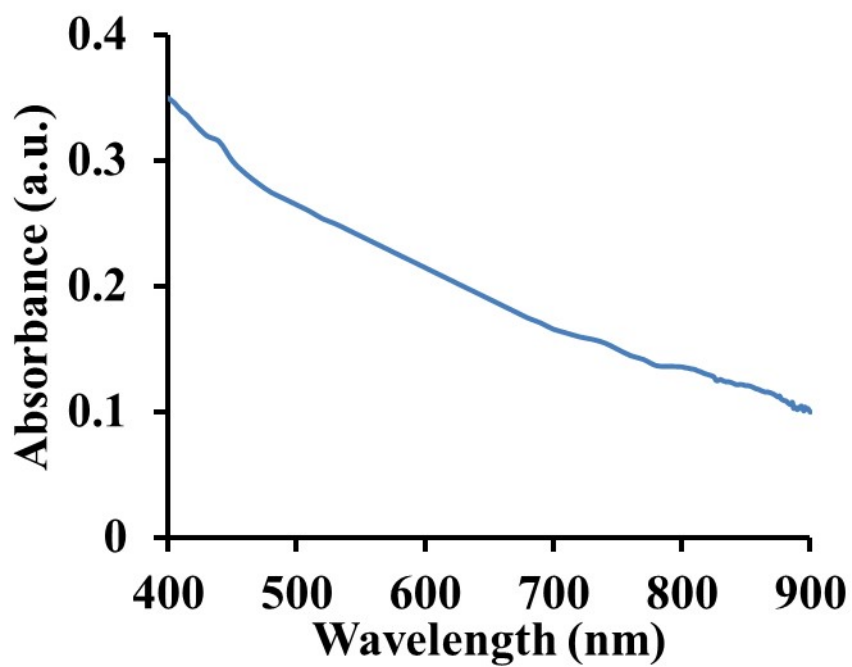
Figure S5. The UV-vis-NIR spectra of Fe$_3$O$_4$@NIPs.
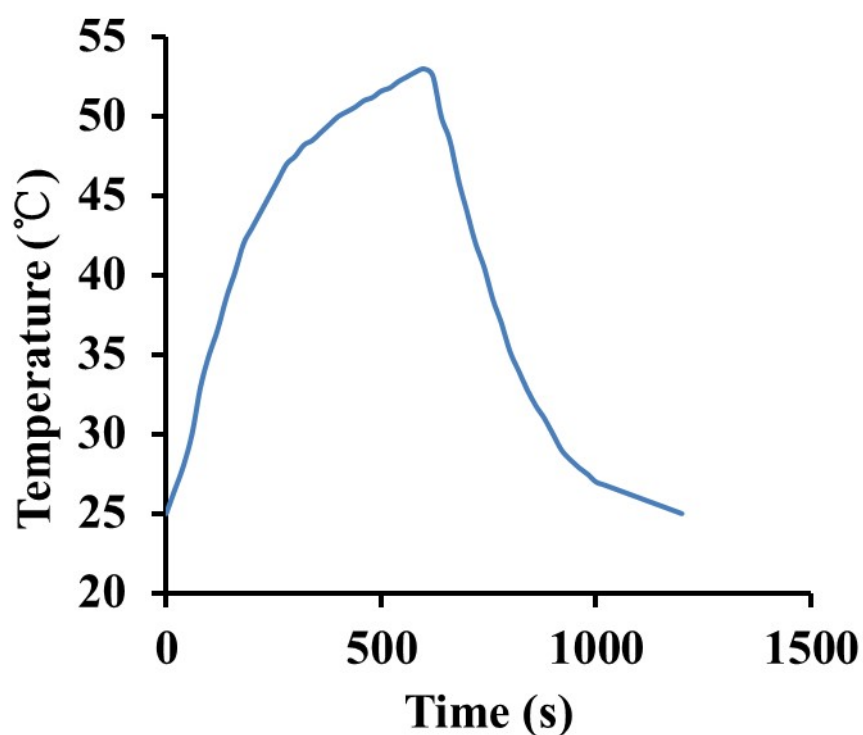
Figure S6. Temperature curves of Fe$_3$O$_4$@NIPs at the concentration of 300 μg mL^{-1} as a function of laser irradiation time.
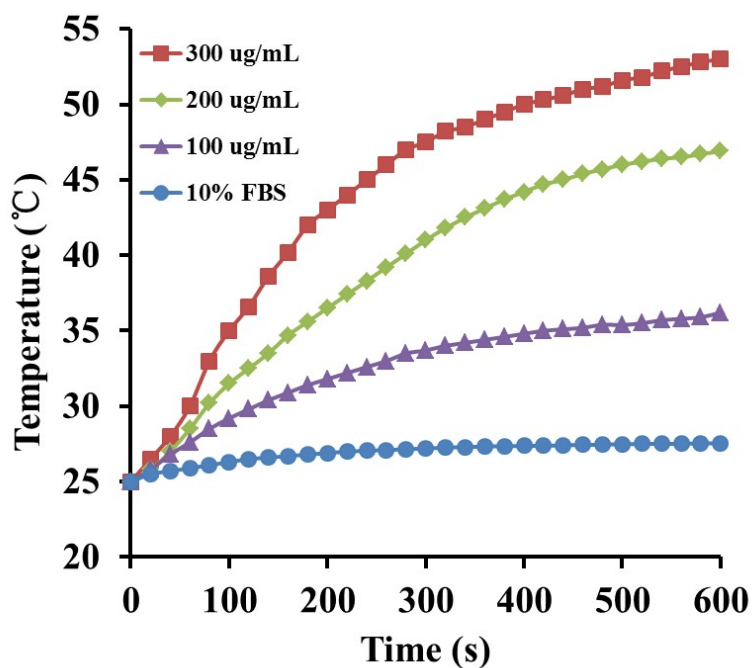
Figure S7. Temperature changes of the solutions containing Fe$_3$O$_4$@NIPs at various concentrations under the irradiation of a 808 nm laser (2.0 W cm^{-2}).
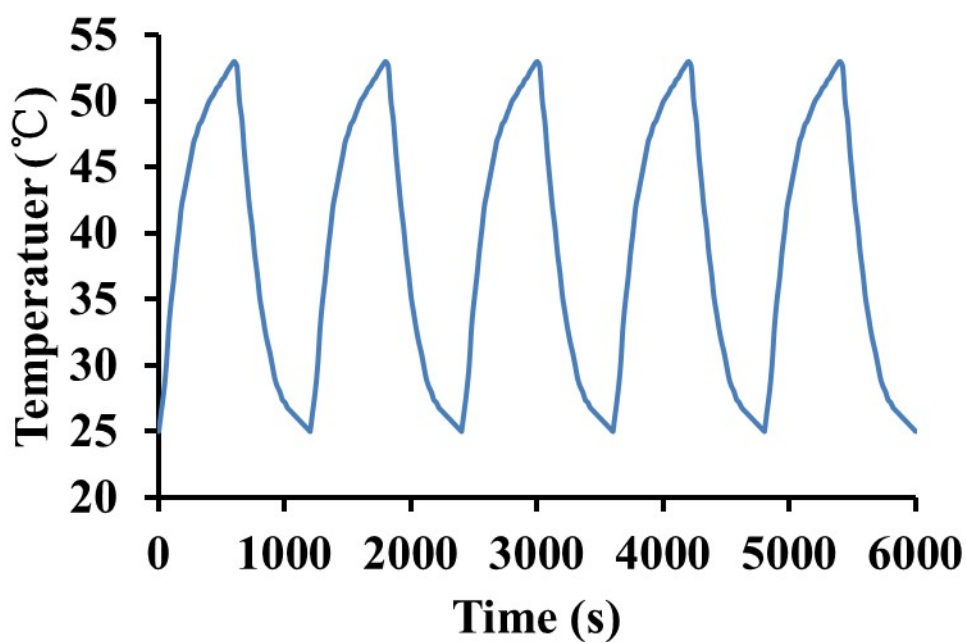
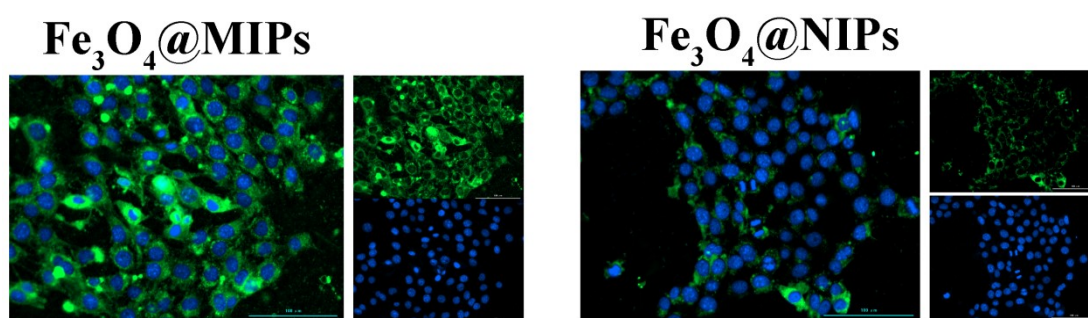Figure S8. Temperature variation of Fe_3O_4@NIPs (300 µg mL^{-1}) under repeated NIR laser irradiations.

Figure S9. Confocal laser scanning microscopy images of 4T1 cells incubated with Fe_3O_4@MIPs and Fe_3O_4@NIPs after 6 h.
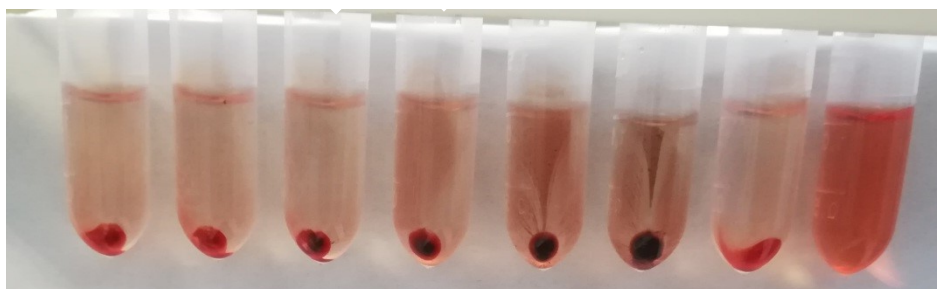
10 μg/mL 25μg/mL 50μg/mL 100μg/mL 200μg/mL 300μg/mL PBS Water

Figure S10. The photographs of erythrocytes after treatments with water (positive control), PBS (negative control), and PBS containing Fe_3O_4@MIPs at different concentrations for 2 h, followed by centrifugation.
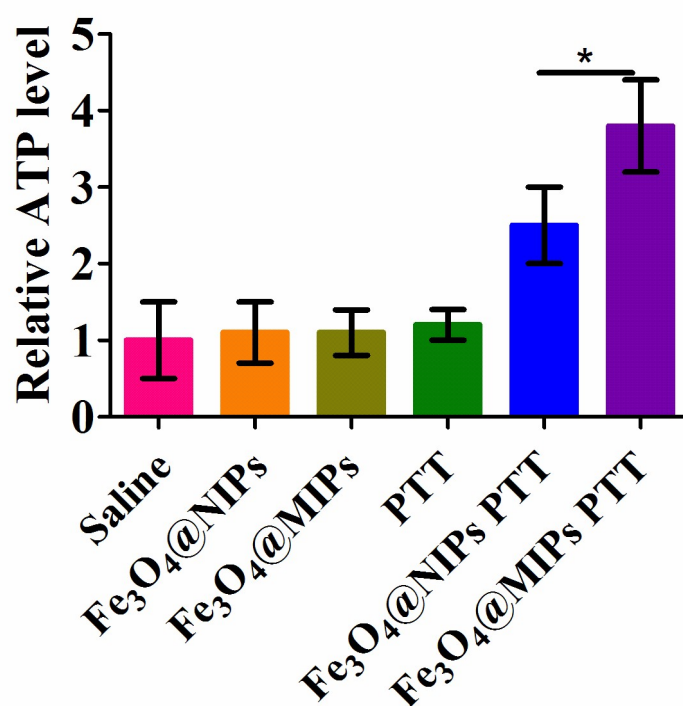

Figure S11. ATP levels in tumor tissues of 4T1-tumor-bearing BALB/c mice after systemic administration of saline, Fe_3O_4@MIPs, or Fe_3O_4@NIPs through tail-vein injection with or without laser irradiation.
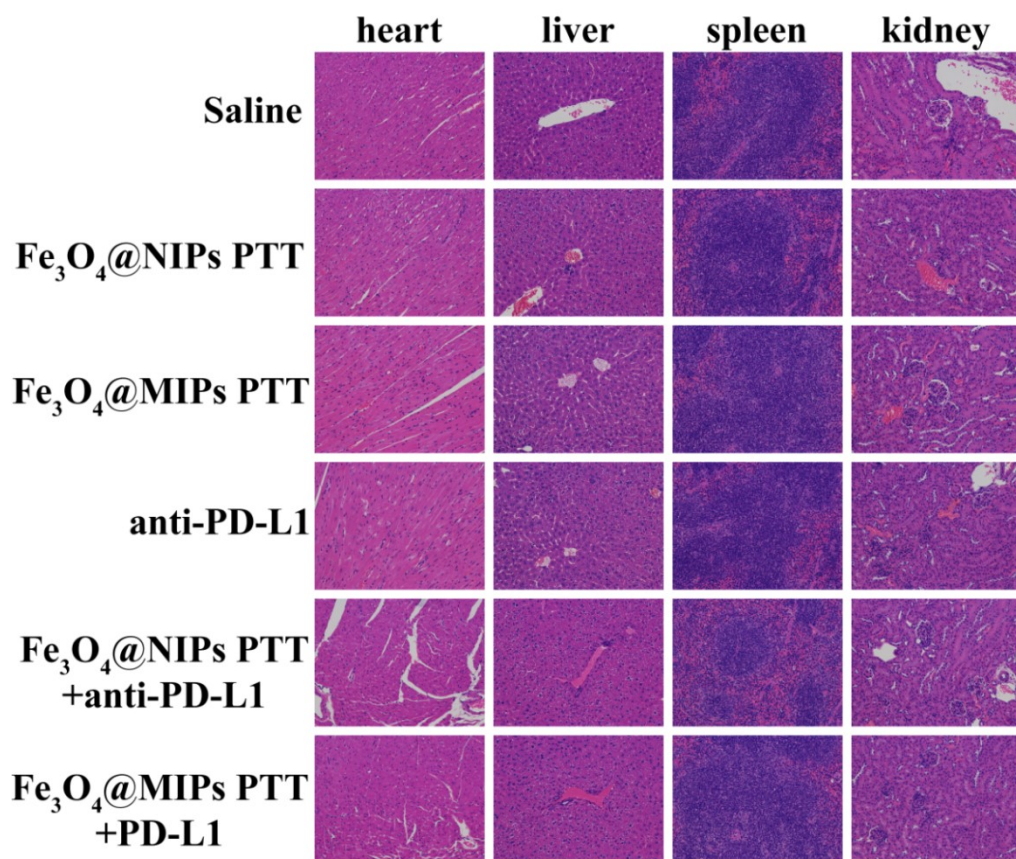
Figure S12. H&E staining in representative tissue sections of mice after treatments.